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Effect of Age on Immune Function in Terms of Chemically Induced Cancers

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Neonatal, fetal, and very old animals are particularly sensitive to chemical carcinogenesis. Reasons for this increased sensitivity could be due to increased susceptibility of "target" organs or cells, peculiar hormonal levels at these age groups, relatively deficient immune functions, or combinations of these and/or other factors. During the late fetal and first three weeks of neonatal life, the immune system is rapidly maturing, is relatively incompetent, and its diverse components are developing at different rates. For example, thymus-dependent (T) alloreactive cells capable of proliferating in mixed lymphocyte reactions (T helper cells) develop by 7 days of age, but precursors of T killer cells are not competent until approximately 14 days of age. Bursa equivalent-dependent (B) cells capable of generating antibody responses are present in fetal liver but are extremely sensitive to tolerance induction until 10-14 days of age when IgD cell surface receptors are detectable. Marrow-dependent (M) cells responsible for regulation of suppressor cells and for natural cytotoxicity to transformed tumor cells do not mature until 3 weeks of age. In very old animals, the thymus is atrophic and cell-mediated immunity is moderately suppressed. Natural cytotoxicity against tumor cells is less than normal but antibody formation (B cell function) is adequate.

Gonadotrophic hormones of the pituitary or placenta are high during pregnancy, the early neonatal period, after the menopause, and in a large fraction of men over 60 years of age. These and other hormones are immunosuppressive and could theoretically facilitate carcinogenesis. The particular immune cell type, if any, responsible for resistance to chemically induced tumors has not been determined. One can only state that susceptibility to chemical carcinogenesis is associated with a relative dysfunction of the immune system and that age is an important factor.

Introduction

Chemical carcinogens and even chemical tumor-promoting agents result in a higher frequency of tumors with shorter latency periods when administered transplacentally to fetuses and during the neonatal period (*1–5*). The occurrence of spontaneous tumors and susceptibility to tumor induction is also increased in old age (*6, 7*). I will describe highlights of what is known about the effect of age on immune function in this review and how this may relate to the age-associated susceptibility to chemically induced cancers.

Ontogeny of the Immune Response

Myelopoietic System

Totipotential hemopoietic stem cells are first detected in the yolk sacs of developing mice on about the eighth day of a 21-day gestation period (*8*). Yolk sac stem cells, when transplanted into lethally irradiated adult mice, differentiate into hemopoietic cell types seen in adult mice, not fetuses (*8*). Therefore, the hemopoietic inductive microenvironment (*9*) differs in the developing animal and regulates the mode of differentiation of stem cells. For example, granulocytes far outnumber macrophages in adult mice but are produced only in small numbers by the fetus. The yolk sac secretes a "colony-stimulating factor" (CSF) which acts on a myeloid precursor to induce differentiation into macrophages primarily (*10*). In adult mice, CSF produced primarily by macrophages (*11*), induces differentiation of the same precursor cell primarily into granulocytes (*10*). Thus, two events must occur before "maturation" of a hemopoietic function occurs: the stem cell or precursor cell must be produced and the hemopoietic "environment" (cell-to-cell or humoral factor) must be appropriate.

Regulation of erythropoietic differentiation is under similar control. During gestation, fetal hemoglobin is produced, but shortly after birth

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transition to the production of adult hemoglobin occurs (*12*). The erythropoietic hormone, erythropoietin, is activated by reticuloendothelial cells of the liver until two weeks after birth when renal glomerular cells take over that function (*13*).

The "immune" function of the myelopoietic system includes phagocytosis, antibody-dependent cellular cytotoxicity and lysis of tumor cells by non-specifically "activated" or "armed" macrophages. The first line of host defense against most extracellular bacteria involves phagocytosis and lysis by granulocytes. This function reaches the "adult" level by about 11 days of age in a representative experiment (*14*). The development of macrophage functions in host defenses appears to depend upon the organism tested. For example, macrophages capable of defense against *Brucella abortis* are mature by the first week of life (*15*), whereas macrophages which protect against Herpes simplex virus-1 hepatitis are immature at 3 weeks of age and do not mature completely until 8 weeks of age (*16*).

Cells with Fc receptors potentially capable of mediating antibody-dependent cellular cytotoxicity (ADCC) are present in the yolk sac and placenta of mice (*17*) and could presumably lyse antibody-coated tumor cells. In humans ADCC is not normal until 3-12 months of age (*18*). No age-related studies of "arming" of macrophages by stimulated lymphocytes have been performed.

Lymphoid System

The thymus gland undergoes organogenesis in the first trimester of human gestation (*19*). Lymphocytes of the thymus were thought to be derived from migrant stem cells from the yolk sac, although recently evidence for an "embryonic" source has been obtained (*20, 21*). The thymus is the central lymphoid organ for cells involved as "helper cells" (T_H) in both humoral and cellular immunity, as "killer cells" (T_K) in cellular immunity and as "suppressor cells" (T_S) in humoral and cellular immunity. T_K cells have been shown by numerous investigators to lyse tumor cells and are of potential great importance in "immunological surveillance" against chemically induced cancers (*19*).

When cells are taken from animals and tested *in vitro* or *in vivo* by adoptive transfer into irradiated adult recipients, T_H cells capable of functioning in humoral antibody responses were detected by 4 days of age (*22*). T_H cells capable of responding (proliferative component of cellular immunity) to *H-2* allogeneic cells *in vivo* or *in vitro*, as above, were detected within 7 days after birth (*23, 24*). T_K cells capable of lysing allogeneic target calls after *in vitro* stimulation in cell-mediated lympholysis (CML) assays are detectable by 14 days of age. Optimal results are obtained in the CML system by collaboration between T_K and T_H cells responding to different types of transplantation antigens (*25*); it has not been determined if precursors of T_K are lacking in spleens of mice less than 2 weeks of age or if T_K–T_H cell interactions are suboptimal.

Suppressor cells for antibody reactions are quite abundant in spleens of neonatal mice (*26–28*). At least some of these suppressors are T_S cells, since they can be inhibited to some extent by antibodies directed against antigens restricted to T cells. T_S cells could explain the ease with which transplantation tolerance can be induced in neonatal mice (*29*). After 3 weeks of age, suppressor cells no longer are so easily detected. We have detected T_S susceptible to activation by Friend leukemia virus in neonatal mice (*30*). Human cord blood cells can suppress the response of maternal lymphocytes *in vitro* (*31*).

Using intact animals or fetuses to detect immune reactions, graft-versus-host reactions by adult lymphocytes can occur in embryonic chickens (*32*), whereas antibody responses are not detectable. Mice (especially C57BL-strain) can reject skin allografts applied during the first few days of life (*33*). However, F1 hybrid mice less than three weeks of age are highly susceptible to lethal graft-versus-host reactions induced by parental-strain lymphoid cells (*34*).

The mammalian equivalent of the bursa of Fabriscius has not been determined; the central lymphoid organ for B cells is definitely not the bone marrow (*35*). B cells are first detected in the fetal liver of mice and B cells capable of generating antibodies upon cell transfer are detectable at 17 days of gestation in the mouse (*22*). However, T_H + B cell collaboration on cell transfer is not mature until 6 days after birth. The sequence of immunoglobulins secreted during ontogeny is IgM, IgG and IgA in most species studied (*36*). The cell surface Ig receptors for antigen also undergo a sequence from IgM to IgM + IgD to IgD + IgG to IgG (*37*). The B cells do not have surface IgD until 10-14 days of age; prior to that time, B cells are extremely susceptible to tolerance induction *in vitro*. Acquisition of IgD makes B cells less easily tolerized. In a recent study, IgE antibody responses were demonstrable in two-week old mice whereas IgM and IgG responses were delayed to the same antigen (*38*). Mice less than three weeks of age not competent for certain humoral and cell-mediated immune reactions (*39*), and are susceptible to tolerance induction (*29*). This is due in part to intrinsic susceptibility of B cells to tolerance, frequency or numbers of responsive B cells (*40*), and an abundance of suppres-

sor cells (*26–28*).

Marrow-dependent (M) cells are a class of immune cells peculiarly dependent upon the integrity of the marrow microenvironment for their differentiation (*35*). Treatment of mice with ^{89}Sr, a long-lived bone-seeking isotope that emits high-energy beta rays, results in aplastic bone marrow. The spleen takes over stem cell functions of the mouse and generates T cells, B cells, macrophages and myeloid cells. Certain immune functions are absent and suppressor cells are increased in number (*30, 35*). The numbers of natural killer (NK) cells capable of lysing tumor cells (particularly hemopoietic tumors) are decreased in adult mice treated with ^{89}Sr (*41*). Such mice are incapable of rejecting hemopoietic allografts (*35*) and C57BL mice treated with ^{89}Sr lose their genetic resistance to leukemia induction and immunosuppression induced by Friend virus (*30*). Mice treated with ^{89}Sr are also more sensitive to infection with the intracellular organisms, Herpes simplex-1 virus and *Listeria monocytogenes* (*14, 42*). M cells do not mature until 3 weeks of age; their functional maturity is associated with a decrease in the numbers of suppressor cells capable of inhibiting antibody responses (*28*) and capable of mediating Friend virus induced immunosuppression (*30*). Mice develop resistance to leukemia virus at 3 weeks of age when M cells are functionally mature (*30*). M cell functions (e.g., NK cell function) are increased in nude mice (*43*), suggesting that T_S cells may inhibit M cells.

The overall balance between various T cell subpopulations results in an increased susceptibility of athymic "nude" mice to polyoma virus induced tumors (*44*) but no increased susceptibility to methyl cholanthrene induced skin tumors (*45*). Nude mice have an increased "natural resistance" to certain infections (*46*), presumably due to an increased number of "activated macrophages" and have "tumoricidal macrophages" (*47*). The macrophages are not "activated" if nude mice are germ-free, a finding which suggests that low-grade infections may be beneficial (*48*).

Aging of the Immune Response

Myelopoietic System

Hemopoietic stem cells serially transplanted into lethally irradiated mice undergo cellular "aging" and lose the ability to protect irradiated mice. The mechanism of this decline in stem cell function is excessive demands on the stem cell for differentiation (*49*). However, myelopoietic stem cells of mice not subjected to such extreme demands for hemopoiesis are functionally competent in aged mice (*12*). Aged individuals are particularly susceptible to fatal bacterial pneumonias following viral infections (*50*). Conceivably, opsonic phagocytosis is impaired in old age. Carbon particle clearance is deficient in old rats (*51*).

Lymphoid System

The thymus begins to involute shortly after sexual development at puberty and steadily decreases in size, number of lymphocytes, and content of the thymic hormones (*52, 53*). Thymus-dependent antibody responses, mitogenic responses to phytohemagglutinin and Concanavalin A and responses to allogeneic cells in mixed lymphocyte cultures decline at approximately the same rate with time after puberty (*54, 55*). However, the decline of T cell functions have been primarily detected in the spleen. Lymph node T cell functions remain vigorous even in aging autoimmune strain NZB mice (*56*). Splenectomy actually prolonged the life of mice in one study (*57*). The loss of T suppressor cells with age is thought to contribute to the high incidence of autoantibodies detectable in the aged population (*52*). The relatively greater loss of certain subpopulations of T cells is thought to also be a contributing factor for the increased incidence of tumors with advancing age (*58*). However, whereas there was a 4-fold decline in cell-mediated immune function in old mice, there was a 100-fold loss of resistance to allogeneic tumor cells (*59*). Thus, other factors may be more important than effector T cells.

There appears to be a relative increase in phagocytic accessory cells in spleens of old mice and this could lead to the observed inability to respond to low doses of antigenic stimulation (*54, 58*). Accessory cells do not lose the capacity to interact with T and B cells in antibody responses with age (*60*).

B cell functions do not deteriorate with age but autoantibodies are produced by B cells in old age (*52*). The consensus of opinion is that loss of T_S cells is responsible for this phenomenon. However, autoimmune potential appears to reflect a marrow stem cell abnormality (*60*) that is corrected by normal marrow but not by normal thymus cells (Jane I. Morton, personal communications). It is therefore conceivable that B cell "excesses" may contribute to the increased susceptibility of old animals to tumors. Splenic B suppressor cells do accumulate with age (*61*).

Marrow-dependent (M) cell functions have not been well investigated in old animals. The ability to reject marrow cell allografts is present in aged mice (unpublished observations). However, the func-

tional activity of natural killer (NK) cells does decline sharply after 8 weeks of age. The peak of activity is detected between 5 and 8 weeks of age (*45, 62*). NK cell function is therefore declining while T and B cell functions (humoral and cellular immunity) have not yet fully matured! The M cell-mediated genetic resistance of C57BL mice to the leukemogenic and immunosuppressive effects of Friend virus persists for many months of age (unpublished observations). The appearance of suppressor cells for the mixed lymphocyte reaction in aging mice is under genetic control and the C57BL mice are "resistant" to the appearance of such suppressor cells (*55*).

Endocrine Status with Respect to Age and Immunity

The relationship between the endocrine status and immunity and tumor susceptibility deserves examination, particularly since transplacental carcinogenesis is so "effective" and the hormone status is so perturbed during pregnancy. During pregnancy in humans, there is a dramatic increase in the level of chorionic gonadotrophic hormones during the first trimester when the allogeneic blastocyst is implanting itself and when organogenesis is occurring rapidly (*63*). Chorionic gonadotrophins are immunosuppressive (*64–66*). Many chemical carcinogens are immunosuppressive (*19*), and the combination of hormones and carcinogen could be quite immunosuppressive. The direct-acting carcinogen 1-ethyl-1-nitrosourea (ENU) is particularly efficient when administered to *Erythrocebus patas* monkeys during the first trimester (*67*). Other hormones increased during pregnancy, e.g., cortisol and progesterone, are immunosuppressive (*68, 69*). The host endocrine status can influence the susceptibility of mice to chemical carcinogens and certain carcinogens can affect the endocrine status (*70*). The ontogeny of the endocrine system could be altered by the induction of immunological transplantation tolerance in neonatal mice not subjected to a graft-versus-host reaction (*71*). Thus, the endocrine and immune system may be closely controlled. A gene regulating testosterone metabolism is linked to the major histocompatibility complex (*H-2*) of the mouse (*72*). Finally, during differentiation of T cells, the ability to recognize "self" versus "non-self" with respect to the ability to respond normally to virus infections is determined by the thymic epithelial cells and not other cells of the body (*73*). These epithelial cells are the source of the thymic hormones.

In aging humans, there is an increase in gonadotrophins after the menopause and in a fraction of men over 60 years of age (*63*). The hypothalamic threshold to feedback control is increased with age (*74*) and this may contribute to endocrine (and immune?) abnormalities. The pituitary begins to secrete a hormone at puberty that inhibits target cell responses to hormones (*51*) and could help mediate immune deficiency in old age.

In summary, one can state that susceptibility to chemical carcinogenesis is associated with relative dysfunctions of the immune system and that age is important because it affects immune function. The effect could be indirect, mediated by hormonal influences. This could explain the high frequency of tumors induced in offspring by carcinogens administered during pregnancy and the occurrence of tumors in the aged.

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